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Host-parasite interactions and immunity to irradiated sporozoites

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1. Summary

We compare and contrast the results of immunizing mice with irradiated sporozoites of *Plasmodium berghei* and *Plasmodium yoelii*. Host genetic control of protective immunity is different in the two rodent malarias. Few mouse strains are strongly protected by *P. yoelii* sporozoites, while all are protected by *P. berghei* sporozoite immunization. The role of $CD8^+$ T cells in the protective immune response to each of these malarias varies with the strain of mouse. Moreover, a single strain will use a $CD8^+$ T cell-dependent mechanism against one malaria, and a $CD8^+$ independent mechanism against the other. Thus, each host-parasite pairing in these rodent malarias engenders a unique set of immune responses. Such variety should be expected in the immune response to the human malarias, and may complicate the development of universally applicable vaccines.

2. Introduction

Vaccination with radiation-attenuated malaria sporozoites can provide sterile immunity against huge sporozoite challenges. This is true in rats and mice [1] as well as humans [2]. The immune responses of these laboratory animals have been intensively studied with the hope that the knowledge gained could be used to devise a synthetic human vaccine. The preconception underlying this approach is that irradiated sporozoites induce a common constellation of protective immune responses in all hosts. I would like to review recent evidence which contradicts this idea. I shall focus on the immune responses to the sporozoites of *P. yoelii* and *P. berghei* in different strains of mice. It now appears that there is no single pattern of immune responses by which mice are protected by irradiated sporozoites. Rather, the different rodent malarias interact with different mouse strains in unique ways. While this does not negate the importance of the rodent malaria models, it does make extrapolation to human vaccines more complex.

Key words: *Plasmodium yoelii*; *Plasmodium berghei*; Sporozoite; $CD8^+$ cells; Immunogenetics

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3. Genetic control of immunity

When inbred strains of mice are immunized with irradiated sporozoites and then infected with live sporozoites to assay for protection, the differences between *P. berghei* and *P. yoelii* are most obvious (Table 1). All strains of mice can be protected by immunization with *P. berghei* sporozoites [3]. The number of immunizing doses may vary from 1 for BALB/c to 4 for C57BL/10 but all strains attain close to complete protection. C57BL/10 congenic mouse strains, identical except for carrying different immune response (Ir) gene alleles, are all protected by irradiated *P. berghei* sporozoites. Although this does not imply that all these strains are being protected by identical responses to the same parasite an-

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TABLE I

Genetic control of immunity to *P. yoelii* and *P. berghei* sporozoites: percentage of mice developing a blood stage infection after homologous immunization and challenge (data adapted from [3, 4]).

	P. yoelii	*P. berghei*
BALB/c	4	0
BALB.B	81	nt
BALB.K	86	nt
B10.D2	89	0
B10	85	0
B10.BR	69	22
B10.S	100	22
B10.M	100	10
B10.RIII	100	0
B10.Q	19	0

tigens, the uniformity of protection is striking.

In contrast, protective immunity induced by irradiated *P. yoelii* sporozoites is variable and genetically controlled [4]. In no mouse strain are all animals protected after a single dose of sporozoites. BALB/c mice require 2 or 3 doses to achieve 100% protection. However, few C57BL/10 mice are protected after even 4–6 doses of *P. yoelii* sporozoites. Interestingly, among the C57BL/10 congenic strains, some are well protected and some are not, showing a degree of Ir gene control not seen in response to *P. berghei* immunization. Highly or poorly protected strains are also seen among congenic strains on the BALB/c background. Furthermore the same Ir gene alleles (H-2d) are associated with high or low degrees of protection depending upon whether the background genes are BALB or C57BL. Thus the efficacy of irradiated *P. yoelii* sporozoites as a vaccine is dependent on complex interactions controlled by mouse Ir and background genes.

4. Differences in sporozoite infectivity

From the genetic analysis it appears that *P. berghei* and *P. yoelii* are interacting with the mouse immune system in strikingly different ways. The reasons for this are not understood. However, it is possible that differences in infectivity between sporozoites of the two malarias may explain these differences in host responses. Dissected *P. yoelii* sporozoites are highly infectious when given i.v. to mice. Typically, an inoculation of 20 sporozoites will give half of all animals a blood stage infection, and 100 sporozoites insures 100% parasitemia in naive animals (W. Weiss, personal observation). There is very little variation between mouse strains: when naive BALB/c, B10.D2, and B10.Q mice were injected with *P. yoelii* sporozoites the 50% infective dose was in the interval between 8–40 sporozoites for all three strains of mice [4].

The infectivity of dissected *P. berghei* sporozoites for mice is much lower. Most published studies use challenge doses of 1000–2000 *P. berghei* sporozoites as this is the minimum number which will reliably infect naive control animals. This might only be a sign that *P. berghei* sporozoites are fragile and easily damaged during dissection. Indeed, infection of mice by mosquito bite gives more consistent infections than do dissected parasites (M. Hollingdale, personal communication). However, a comparison of inbred mice has shown enormous strain variability, up to several orders of magnitude, in the ease of infection by dissected *P. berghei* sporozoites (D. Gordon et al., in preparation). Thus factors other than sporozoite fragility may contribute to the low infectivity of *P. berghei* in mice.

The laboratory mouse is not the natural host of either *P. berghei* or *P. yoelii*. The above data on infectivity may indicate that *P. yoelii* is better equipped to invade and develop in the "foreign" mouse host than is *P. berghei*. If most *P. yoelii* sporozoites penetrate into liver cells, then a specific array of parasite antigens, perhaps quite limited, will be presented to the host immune system on the hepatocyte surface [5]. As normal hepatocytes have few MHC class I molecules and no MHC class II molecules on their surface, the presentation of parasite antigens may be further restricted. On the other hand, if most *P. berghei* sporozoites do not invade hepatocytes, they will die and be scavanged by the reticuloendothelial system, where a different and perhaps much larger set of parasite antigens will be presented to the host in the context of both MHC class I and II molecules. Thus the genetic control of protection by Ir and background genes seen in *P. yoelii* could be due to restricted responses to only a few hepatic stage antigens. *P. berghei* sporozoite immunization would present the host with a larger number of parasite antigens, which might induce a variety of liver specific and systemic immune responses. In effect, the deft

P. yoelii sporozoites are facile at "hiding" from the immune response inside the hepatocyte, while the inefficient *P. berghei* sporozoites blunder into the mouse immune system. It is likely that in highly evolved pairs of host and *Plasmodia*, which may include man and the human malarias, infectivity will be high and immunity to irradiated sporozoites will rest on a very narrow immunologic base. Whether or not this theory is correct, the genetic controls on *P. yoelii* and *P. berghei* sporozoite immunizations are divergent, and illustrate the variability of the immune responses involved.

5. The variable importance of CD8$^+$ T cells in immunity to sporozoites

The unique qualities of the immune response in each pairing of host and parasite is further illustrated by the variable role of CD8$^+$ T cells in sporozoite immunized mice. Initially, it appeared that all mice immunized with sporozoites required CD8$^+$ T cells for protection against sporozoite challenge (Table 2). Depletion of CD8$^+$ T cells by the injection of anti-CD8 monoclonal antibodies eliminated protective immunity to *P. berghei* [6] and *P. yoelii* [7] sporozoites. Now there is evidence that, depending on the strain of mouse, CD8$^+$ T cells may or may not be critical effectors, and a second immune effector arm may protect the animals [4]. This was discovered when mice of BALB/c, B10.BR, and B10.Q strains were immunized with *P. yoelii* sporozoites and depleted of CD8$^+$ T cells (Table 3). The BALB/c mice lost their immunity but the B10.BR and B10.Q mice remained protected. This implies an effector arm independent of CD8$^+$ T cells in these strains which is adequate to protect. This second mechanism is not protective in BALB/c mice nor indeed in the other B10 congenic mice which were not well protected by sporozoite immunization.

TABLE 2

The effect on sporozoite immunized mice of T cell depletion by the injection of anti-T cell antibodies. Data show the number developing parasitemia/total challenged with sporozoites (adapted from [6, 7]).

Plasmodium species	Mouse strain	In vivo depletion	Infected/total
P. berghei	A/J	none	0/10
		anti-CD8	5/5
		anti-CD4	0/5
P. yoelii	BALB/c	none	0/10
		anti-CD8	9/9
		anti-CD4	0/5

TABLE 3

The effect of depleting CD8$^+$ T cells on protection in mice immunized with *P. yoelii* sporozoites. Data is presented as animals with parasitemia/total challenged (adapted from [4]).

	Immune + anti-CD8	Immune + control mAb	Naive mice
BALB/c	10/10	0/10	5/5
B10.BR	4/ 9	2/ 9	5/5
B10.Q	2/ 8	1/ 8	6/6

Similar evidence of a second effector mechanism independent of CD8$^+$ T cells and limited to certain mouse strains has now been seen in *P. berghei* (Table 4) (M. Sequin et al., in preparation). As previously described [6], A/J mice immunized with *P. berghei* sporozoites are not protected after CD8$^+$ T cell depletion. However, BALB/c mice immunized with *P. berghei* sporozoites remain resistant to infection after CD8$^+$ T cells are depleted. Thus, just as in the *P. yoelii* example, certain strains of mice mount a protective immune response dependent on CD8$^+$ T cells, while other strains have an alternative mechanism of protection. Yet the choice of mechanism depends upon the specific host-parasite pair, as BALB/c mice are protected against *P. yoelii* by the CD8$^+$-dependent mechanism and by the CD8$^+$-independent mechanism against *P. berghei* sporozoites. It appears that every pairing of mouse strain and sporozoite generates its own pattern of protec-

TABLE 4

Results of treating sporozoite immunized mice with anti-CD8 mAb prior to challenge (summarized from M. Sequin et al., in preparation).

Mouse strain	Immunizing sporozoite	Control Ab treatment	Anti-CD8 treatment
A/J	*P. berghei*	immune	not immune
BALB/c	*P. berghei*	immune	immune
BALB/c	*P. yoelii*	immune	not immune

tive immune responses. For *P. berghei*, all mice can be protected but utilize different immune effector mechanisms depending on the strain of mouse. For *P. yoelii* there is a similar variability in the induced effector mechanisms depending on the mouse genetic background but not all strains of mice are protected.

6. Human vaccines

I believe that a human vaccine which successfully protects against sporozoite challenge will have to reproduce the formidable protective immune responses produced by irradiated sporozoites. Given the heterogeneity of responses to irradiated sporozoites in mice, it seems unlikely that a single dominant immune response will protect all humans against malaria. Variations should be expected between human *Plasmodium* species or among different human populations. In particular, we should be wary of generalizations based on data from any one host-parasite combination. Weak immunity may be achieved by activating immune effector mechanisms other than those which dominate the response to irradiated sporozoites. For example, after $CD8^+$ T cell depletion in BALB/c mice neither serum antibodies nor $CD4^+$ T cells are sufficient to protect against *P. yoelii* sporozoites [7]. Yet transfer of monoclonal antibodies gives transient protection (V. Charoenvit et al., in preparation), and $CD4^+$ T cell clones can protect naive BALB/c mice against small numbers of sporozoites [8]. When compared to sporozoite-induced protection these alternative immune mechanisms are deficient in duration or extent. Perhaps further work will produce more potent vaccines which work by immune mechanisms different from those activated by irradiated sporozoites. However, despite their complexity, we should strive to understand dominant host-parasite responses, and design vaccines for humans which activate the major protective pathways. Otherwise we may never achieve the level and duration of immunity which will be required of a successful preerythrocytic malaria vaccine in the field.

References

[1] Nussenzweig, R. S., Vanderberg, J., Most, H. and Orton, C. (1967) Nature 216, 160.

[2] Clyde, D. F., Most, H., McCarthy, V. C. and Vanderberg, J. P. (1973) Am. J. Med. Sci. 266, 169.

[3] Hoffman, S. L., Berzofsky, J. A., Isenbarger, D., Zeltzer, E., Majarian, W. R., Gross, M. and Ballou, W. R. (1989). J. Immunol. 142, 3581.

[4] Weiss, W. R., Good, M. F., Hollingdale, M. R., Miller, L. H. and Berzofsky, J. A. (1989). J. Immunol. 143, 4263.

[5] Weiss, W. R., Mellouk, S., Houghten, R. A., Sedegah, M., Kumar, S., Good, M. F., Berzofsky, J. A., Miller, L. H. and Hoffman, S. L. (1990). J. Exp. Med. 171, 763.

[6] Schofield, L.,Villaquiran, J., Ferreira, A., Schellekens, H., Nussenzweig, R. and Nussenzweig, V. (1987). Nature 330, 664.

[7] Weiss, W. R., Sedegah, M., Beaudoin, R. L., Miller, L. H. and Good, M. F. (1988) Proc. Natl. Acad. Sci. USA 85, 573.

[8] Grillot, D., Michel, M., Muller, I., Tougne, C., Renia, L., Mazier, D., Corradin, G., Lambert, P.-H., Louis, J. A. and Del Giudice, G. (1990) Eur. J. Immunol., in press.

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